Masking of the background fluorescence and luminescence in the optical analysis of biomedical assays

Abstract

In a process for the quantitative optical analysis of fluorescently labelled biological cells 5, a cell layer on a transparent support at the bottom 2 of a reaction vessel 1 is in contact with a solution 3 containing the fluorescent dye 4. The sensitivity of analytical detection can be considerably improved if to the fluorescent dye 4 already present in addition a masking dye 9, which absorbs the excitation light 6 for the fluorescent dye 4 and/or its emission light 7, is added to the solution 3 and/or if a separating layer 10 permeable to the solution and absorbing and/or reflecting the excitation light 6 or the emission light 7 is applied to the cell layer at the bottom 2. This process can also be used for improving the sensitivity in the quantitative optical analysis of a luminescent biological cell layer. The separating layer 10 must in this case be composed such that it has a high power of reflection for the luminescent light 11. Analogously, these process principles can also be used in receptor studies for the masking of the interfering background radiation in the quantitative optical analysis of fluorescently or luminescently labelled reaction components. In this case, a receptor layer 12 at the bottom 2 of a reaction vessel 1 is in contact with a solution (supernatant 3) in which a fluorescent or luminescent ligand 13 is dissolved. The sensitivity and accuracy of the analytical detection can be considerably improved here if a masking dye 9 which absorbs the excitation light 6 for the fluorescent dye and/or its emission light or (in the case of luminescent ligands) the luminescent light is added to the supernatant 3. Instead of the masking dye in the solution 3 or optionally as an additional measure, a separating layer 10 permeable to the solution 3 and absorbing and/or reflecting the excitation light 6 and/or the emission light or the luminescent light can be applied to the cell or receptor layer 12 at the bottom 2.

(Figs. 2 and 10)